PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 21581 WO-BUR	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/EP2004/000729	International filing date *(day/month/year)* 28.01.2004	Priority date *(day/month/year)* 29.01.2003
International Patent Classification (IPC) or national classification and IPC C07H21/04		
Applicant ROCHE DIAGNOSTICS GMBH et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the International application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.06.2004	29.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Hennard, C Telephone No. +49 89 2399-7355

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/000729

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-26	as originally filed
Claims, Numbers	
1-14	received on 07.09.2004 with letter of 03.09.2004
Drawings, Sheets	
1/14-14/14	as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	None
Inventive step (IS)	Yes:	Claims	1-14
	No:	Claims	None
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	None

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The following documents have been used in the evaluation of the present application:
   D1: NUCLEIC ACIDS RESEARCH, vol. 29, no. 13, 2001, pages e65-1-e65-7
   D2: JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 92, no. 3, 1970, pages 724-726
   D3: ANALYTICAL BIOCHEMISTRY, vol. 226, 1995, pages 161-166
   D4: THE JOURNAL O BIOLOGICAL CHEMISTRY, vol. 257, no. 9, 1982, pages 4796-4805
   D5: BIOTECHNIQUES, vol. 33, no. 3, September 2002, pages 526-531
   D6: NUCLEIC ACIDS RESEARCH, vol. 22, no. 4, 1994, pages 695-696
   D7: NUCLEIC ACIDS RESEARCH, vol. 26, no. 21, 1998 pages 5009-5010
   D8: NUCLEIC ACIDS RESEARCH, vol. 22, no. 15, 1994, pages 2990-2997
   D9: US-A-4 844 880

2. **Novelty (Article 33(2) PCT):**
   The claimed subject-matter of the newly filed **claims 1-14** of the present application are not disclosed in the documents cited and is therefore considered novel. These claims fulfil the requirements of **article 33(2) PCT.**

3. **Inventive merit (Article 33(3) PCT):**
   D1, which is considered to be the closest prior art, concerns the transformation of cytosine into uracil using various operating conditions involving bisulphite as a reactant (see page e65-2, "deamination"; page e65-3, table 1 and last paragraph). In particular, this document describes the bisulphite reaction at 80 and 85 degrees Celsius during 1 and 4 hours (among others) and using bisulphite concentrations between 3.87 - 4.26 M or between 5.20 - 5.69 M at pH 5.0. This document also clearly teaches that by increasing the reaction temperature, the full conversion is achieved in a shorter time.
   The method of the application distinguishes itself from **D1** by the reacting time which is between 1.5 and 3.5 hours.
   From the comparative tests provided by the applicant (with letter of 13.10.2004) it appears that the technical effect achieved by selecting a reaction time between 1.5 and 3.5 hours using the concentration, pH and temperature as defined in **claim 1** is that a higher transformation yield is obtained.

The problem to be solved by the present application can therefore be formulated as to find a method to transform cytidine into uracil with better yield.
The solution suggested by the present application is therefore an alternative to **D1**.
The comparative example presented in the tests of 13.10.2004 demonstrate the unexpected effect that the combination of the specific conditions (concentration of bisulfite, pH, temperature and reaction time) give a higher transformation yield.
Due to this unexpected result, an inventive merit can be recognised in the method of **claim 1** which thus fulfills the requirements of **article 33(3) PCT**.
The optimised conversion conditions being obtained by the combination of the appropriate concentration of bisulfite, pH and the temperature, the use of such a solution for the conversion of cytosine to uracil (**claim 8**) as well as the kit (**claim 11**) and the solution (**claim 12**) claimed are also considered to demonstrate an inventive merit over the prior art.
It is concluded that **claims 1-14** of the present application fulfil the requirements of **article 33(3) PCT**.

4. **Industrial applicability (Article 33(4) PCT):**
Due to the nature of the claims, an industrial applicability of the invention is obvious and **claims 1-14** are considered to fulfil the requirements of **Article 33(4) PCT**.

Enclosure to letter of September 3, 2004
International Patent Application No. PCT/EP04/00729
Applicant: Roche Diagnostics GmbH
Applicant's Ref.: 21581 WO-BUR

New Patent Claims

1. Method for the conversion of a cytosine base in a nucleic acid to an uracil base comprising the steps of

   a) incubating a solution comprising the nucleic acid for a time period of 1.5 to 3.5 hours at a temperature between 70 and 90 °C, whereby the concentration of bisulfite in the solution is between 3 M and 6.25 M and whereby the pH value of the solution is between 5.0 and 6.0 whereby the nucleic acid is deaminated, and
   b) incubating the solution comprising the deaminated nucleic acid under alkaline conditions whereby the deaminated nucleic acid is desulfonated.

2. Method according to claim 1,
characterized in that
in step a) the temperature is between 75 and 85 °C.

3. Method according to any of the claims 1 to 2,
characterized in that
the concentration of bisulfite is between 3.2 M and 6 M.

4. Method according to any of the claims 1 to 3,
characterized in that
the pH value of the solution is between 5.25 and 5.75.

5. Method according to any of the claims 1 to 4,
characterized in that
the time period is between 1.75 and 3 hours.

6. Method according to any of the claims 1 to 5,
characterized in that
the time period is between 2 and 3 hours.

7. Method according to any of the claims 1 to 6, characterized in that in step a) the temperature is 80 °C, the concentration of bisulfite is 5 M, the pH value of the solution is 5.5 and the time period is between 2 and 3 hours.

8. Use of a solution with a pH value between 5.25 and 5.75 comprising bisulfite in a concentration between 3 M and 6.25 M at a reaction temperature between 70 and 90 °C and optionally comprising hydroquinone in a reaction wherein a cytosine base in a nucleic acid is converted to an uracil base in the presence of bisulfite ions...

9. Use according to claim 8 wherein the concentration of bisulfite is between 3.2 M and 6 M.

10. Use according to any of the claims 8 to 9 wherein the pH value of the solution is 5.5 and wherein the concentration of bisulfite is 5 M.

11. Kit comprising a solution with a pH value between 5.25 and 5.75 comprising bisulfite in a concentration between 3 M and 6.25 M and optionally comprising hydroquinone.

12. Solution with a pH value between 5.4 and 5.6 and comprising bisulfite in a concentration between 3.5 M and 6.25 M and optionally comprising hydroquinone.

13. Solution according to claim 12 wherein the concentration of bisulfite is between 3.75 M and 6 M.

14. Solution according to any of the claims 12 to 13 wherein the pH value of the solution is 5.5 and wherein the concentration of bisulfite is 5 M.